

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>BO 42358</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/NL 98/ 00721</b>	International filing date (day/month/year) <b>18/12/1998</b>	(Earliest) Priority Date (day/month/year) <b>18/12/1997</b>
Applicant <b>VAN EELEN, Willem, Frederik et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.  Certain claims were found unsearchable (See Box I).

3.  Unity of invention is lacking (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

**INDUSTRIAL PRODUCTION OF MEAT FROM IN VITRO CELL CULTURES**

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 98/00721

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 6 C12N5/06 A23L1/31

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbol)

IPC 6 C12N A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category <sup>°</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 28738 A (HALDANE FOODS GROUP LTD) 22 December 1994 see abstract ----	1,12, 14-23
Y	WO 94 08598 A (ADVANCED TISSUE SCIENCES INC.) 28 April 1994 see abstract ----	1-5,12, 14-23
Y	US 5 624 840 A (NAUGHTON BRIAN A. ET AL) 29 April 1997 see abstract see column 3, line 65 - column 4, line 46 see column 7, line 55 - column 11, line 28 ----	1-5,12
A	WO 96 40889 A (ST JUDE MEDICAL) 19 December 1996 see abstract see page 6, line 15 - page 7, line 15 ----	3 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

17 March 1999

24/03/1999

Name and mailing address of the ISA  
 European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Panzica, G

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/NL 98/00721

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 00632 A (AMGEN INC) 5 January 1995 see abstract see page 7, line 31 - page 11, line 3 see page 27, line 25 - page 33, line 11 ----	3,4,8,10
A	WO 97 18842 A (FIDIA ADVANCED BIOPOLYMERS SRL) 29 May 1997 see abstract see page 3, line 18 - page 5, line 13 ----	3-5,7-9, 12
A	GB 1 433 841 A (THE QUAKER OATS CO.) 28 April 1976 see the whole document -----	14,15, 18,19,21

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/NL 98/00721

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9428738	A 22-12-1994	AT 157224 T AU 684794 B AU 6934494 A DE 69405235 D DE 69405235 T DK 703729 T EP 0703729 A GB 2294192 A, B JP 8511422 T US 5804246 A	15-09-1997 08-01-1998 03-01-1995 02-10-1997 12-03-1998 14-04-1998 03-04-1996 24-04-1996 03-12-1996 08-09-1998
WO 9408598	A 28-04-1994	AU 689758 B AU 5355494 A CA 2146747 A EP 0671923 A JP 8502175 T US 5559022 A	09-04-1998 09-05-1994 28-04-1994 20-09-1995 12-03-1996 24-09-1996
US 5624840	A 29-04-1997	US 5510254 A US 5443950 A US 5266480 A US 5032508 A US 4963489 A US 4721096 A US 5849588 A US 5460939 A US 5580781 A US 5516680 A US 5512475 A US 5541107 A US 5516681 A US 5578485 A US 5785964 A US 5518915 A US 5863531 A US 5858721 A AU 4211489 A CA 1335657 A DK 40591 A EP 0358506 A IL 91536 A JP 4501657 T NZ 230572 A PT 91676 A WO 9002796 A US 5160490 A AT 127692 T AU 6815990 A AU 6816090 A AU 615414 B AU 7356887 A BG 51337 A CA 1310926 A DE 3751519 D DK 665687 A EP 0309456 A FI 884783 A GR 88100216 A	23-04-1996 22-08-1995 30-11-1993 16-07-1991 16-10-1990 26-01-1988 15-12-1998 24-10-1996 03-12-1996 14-05-1996 30-04-1996 30-07-1996 14-05-1996 26-11-1996 28-07-1998 21-05-1996 26-01-1999 12-01-1995 02-04-1990 23-05-1995 07-05-1991 14-03-1990 31-10-1996 26-03-1992 23-12-1993 30-03-1990 22-03-1993 03-11-1991 15-09-1995 14-03-1991 14-03-1991 03-10-1991 09-11-1987 15-04-1993 01-12-1992 19-10-1995 17-12-1987 05-04-1989 17-10-1988 31-01-1989

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 98/00721

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5624840	A	IL 85957 A JP 10114664 A JP 1503195 T NO 179181 B PT 87136 B RO 106655 A WO 8706120 A AU 595813 B AU 5985086 A	24-06-1994 06-05-1998 02-11-1989 13-05-1996 30-11-1992 30-06-1993 22-10-1990 12-04-1990 22-10-1987
WO 9640889	A	19-12-1996	AU 5990696 A
WO 9500632	A	05-01-1995	US 5405772 A AU 678836 B AU 7112494 A CA 2165335 A EP 0703978 A JP 8508891 T
WO 9718842	A	29-05-1997	IT PD950225 A AU 7693496 A CA 2238011 A EP 0863776 A
GB 1433841	A	28-04-1976	NL 7310162 A
			28-01-1974

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:  
United States Patent and Trademark  
Office  
(Box PCT)  
Crystal Plaza 2  
Washington, DC 20231  
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing:

24 June 1999 (24.06.99)

in its capacity as elected Office

International application No.:

PCT/NL98/00721

Applicant's or agent's file reference:

BO 42358

International filing date:

18 December 1998 (18.12.98)

Priority date:

18 December 1997 (18.12.97)

Applicant:

VAN EELEN, Willem, Frederik et al

1. The designated Office is hereby notified of its election made:

in the demand filed with the International preliminary Examining Authority on:  
27 April 1999 (27.04.99)

in a notice effecting later election filed with the International Bureau on:  
\_\_\_\_\_

2. The election  was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PENT COOPERATION TREA.

09/5B/912  
5000

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)Date of mailing (day/month/year)  
27 June 2000 (27.06.00)

From the INTERNATIONAL BUREAU

To:

DE BRUIJN, Leendert, C.  
Nederlandsch Octrooibureau  
Scheveningseweg 82  
P.O. Box 29720  
NL-2502 LS The Hague  
PAYS-BASApplicant's or agent's file reference  
BO 42358

## IMPORTANT NOTIFICATION

International application No.  
PCT/NL98/00721International filing date (day/month/year)  
18 December 1998 (18.12.98)

1. The following indications appeared on record concerning:

 the applicant  the inventor  the agent  the common representativeName and Address  
VAN EELN, Willem, Frederik  
Broekerwaard 106  
NL-1824 EV Alkmaar  
NetherlandsState of Nationality  
NL  
State of Residence  
NL  
Telephone No.  
Facsimile No.  
Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

 the person  the name  the address  the nationality  the residenceName and Address  
VAN EELN, Willem, Frederik  
Sumatrakade 99  
NL-1019 PJ Amsterdam  
NetherlandsState of Nationality  
NL  
State of Residence  
NL  
Telephone No.  
Facsimile No.  
Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

 the receiving Office  
 the International Searching Authority  
 the International Preliminary Examining Authority the designated Offices concerned  
 the elected Offices concerned  
 other:The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35

Authorized officer

A. Karkachi

Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

REC'D 17 SEP 1999

WIPO PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BO 42358	<b>FOR FURTHER ACTION</b> <small>See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)</small>	
International application No. PCT/NL98/00721	International filing date (day/month/year) 18/12/1998	Priority date (day/month/year) 18/12/1997
International Patent Classification (IPC) or national classification and IPC C12N5/06		
Applicant VAN EELEN, Willem Frederik et al.		
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 4 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p>		
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <li>I <input checked="" type="checkbox"/> Basis of the report</li> <li>II <input type="checkbox"/> Priority</li> <li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li> <li>IV <input type="checkbox"/> Lack of unity of invention</li> <li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li> <li>VI <input type="checkbox"/> Certain documents cited</li> <li>VII <input type="checkbox"/> Certain defects in the international application</li> <li>VIII <input checked="" type="checkbox"/> Certain observations on the international application</li> </ul>		

Date of submission of the demand 27/04/1999	Date of completion of this report 17/09/1999
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Paloniemi Legland, R Telephone No. +49 89 2399 8576



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL98/00721

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-18 as originally filed

**Claims, No.:**

1-23 as originally filed

2. The amendments have resulted in the cancellation of:

the description,      pages:  
 the claims,      Nos.:  
 the drawings,      sheets:

3.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-23
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-23
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL98/00721

**2. Citations and explanations**

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/NL98/00721

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**Novelty (Art. 33(2) PCT):**

A process for production of meat product comprising the culturing in vitro of animal cells in medium free of hazardous substances for humans on an industrial scale thereby providing three dimensional animal muscle tissue followed by further processing steps of the cell culture to a finished food product and a meat product obtainable from such process is not disclosed in the prior art cited in the International Search Report (in particular the exposure of hazardous products and the further processing into a food product is not disclosed). Subject matter of claims 1-23 is therefore regarded to be novel over the prior art.

**Inventive step (Art. 33(3) PCT):**

Closest prior art is regarded to be WO 97/18842. Difference to the present application: there is no further processing into a food product and hazardous substances (antibiotics) are present in this prior art process/product. Problem was to provide meat products not based on animals but on cell cultures of animal cells. This problem was solved by the claimed process. This solution is not regarded as being obvious for the skilled person, because in the prior art the animal cells have been used as research objects and there have not been any hints to use these cells as a food product. Thus subject matter of claims 1-23 is regarded to involve an inventive step.

**Re Item VIII**

**Certain observations on the international application**

The wording "derivable" in claim 17 should be replaced by "obtainable".

Nederlandsch Octrooibureau  
INGEK. 17 OKT 1999  
Paraaf Bewerken

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 05 October 1999 (05.10.99)
Applicant's or agent's file reference BO 42358
International application No. PCT/NL98/00721

From the INTERNATIONAL BUREAU

To:

DE BRUIJN, Leendert, C.  
Nederlandsch Octrooibureau  
Scheveningseweg 82  
P.O. Box 29720  
NL-2502 LS The Hague  
PAYS-BAS

## IMPORTANT NOTIFICATION

International filing date (day/month/year)  
18 December 1998 (18.12.98)

## 1. The following indications appeared on record concerning:

the applicant  the inventor  the agent  the common representative

Name and Address	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person  the name  the address  the nationality  the residence

Name and Address MUMMERY, Christine Hasebroeklaan 93 3723 DJ Bilthoven Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

## 3. Further observations, if necessary:

New applicant/inventor for all designated States.

## 4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Athina Nickitas-Etienne
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 398.83.38

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
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CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

**Title: INDUSTRIAL PRODUCTION OF MEAT FROM *IN VITRO* CELL CULTURES****5      Summary of the invention**

The subject invention is directed at the production of a meat product. The product itself also falls within the scope of the invention.

**Background to the invention**

10      The current world population is exceedingly large and still growing. In order for the population to be fed sufficiently more and more land is required for food produce. The natural sources are insufficient to fulfil the demand. This has led to famine in some parts of the world. In other parts of the world the problem is being addressed by large scale production of animals often under atrocious inhumane conditions.

15      This large scale production is not only causing unnecessary great suffering to animals. It is increasing the number of diseases and the consequences thereof for both animals and humans. Large scale slaughtering is currently required to fulfil the current food requirements and as a consequence of large scale disease outbreaks. We can take for example the recent large scale occurrence of porcine pestivirus and mad cows disease. These diseases also result in loss of the meat for human consumption thus 20 completely denying the purpose for which the animals were being bred in the first place.

25      In addition the large scale production is reducing the flavour of the finished product. A preference exists among those that can afford it for non battery laid eggs and non battery produced meat. Not only is it a matter of taste but also a healthier choice thereby avoiding consumption of various feed additives such as growth hormones.

30      Another problem associated with mass animal production is the environmental problem caused by the enormous amounts of excrement the animals produce and which the environment subsequently has to deal with. Also the large amount of land currently required for animal production or the production of feed for the animals which cannot be used for alternative purposes such as growth of other crop, housing, recreation, wild nature and forests.

A solution has been sought for the above-mentioned problems and is

described in detail below.

Detailed description of the invention:

The subject invention is directed at solving the above mentioned problems by providing a novel production process for meat. The invention also covers the food product itself. The novel process does not require the large scale inhumane production of animals (bio-industry) and in fact does not require the loss of any animal life or animal suffering. The process does however render a product that is healthy and free of growth hormones at levels exceeding physiological levels. The product can replace current meat products.

The subject invention is directed at a process for production of a meat product said process comprising the culturing in vitro of animal cells in medium free of hazardous substances for humans on an industrial scale thereby providing three dimensional animal tissue suited for human consumption, said cells being selected from muscle cells, somite cells or stem cells.

Industrial scale means on a scale other than laboratory scale. Thus the cultivation containers used for cell growth will be larger than 5 litres, and can even be larger than 5000 litres. Preferably this means on a scale for production similar to that applied for example in the production of yeast cells in the food sector or in the penicillin production field about which numerous publications are available to the person skilled in the art.

To date the three dimensional production of muscle cells, in vitro had not been carried out. Three dimensional means in a manner resulting in a composition comprising multiple cells forming one structure in all three dimensions. To date explant cultures whereby tissue was extracted in vivo and placed on a petri dish and cultivated resulted in the growth of monolayers of muscle cells. This process was only carried out on laboratory scale in order to assist in the medical world either in research directed at ascertaining growth related characteristics or directed at medical wound treatment. The explant culture technique has never been used or suggested for application in food production. In fact this technique is not suited for food production as the process mainly is directed at keeping the extracted tissue alive rather than generating large amounts of new muscle cells as a tissue. A description of the explant technique can be found for example in Wounds 1991;3 (3):102-110 an article coauthored by one of the subject

inventors (The authors of the cited article are C. Le Poole, P.K. Das, S.R. Krieg, J.R. Mekkes and W. Westerhof). In medical literature monolayer cultures of striated muscle cells were used to study metabolism and kinetics, in physiological and pathophysiological context. In the literature one can also find descriptions of in vivo culture of muscle tissue in the context of wound healing or partial organ replacement, however, such a method has not been taught or suggested for food production and is in fact not suited therefore. It would not solve the problems already addressed above for conventional meat production.

Contrary to the existing belief that the in vitro culture of three dimensional muscle tissue was impossible due to the inhibitory action exerted by the muscle cells themselves upon contact with other muscle cells the subject inventors have found a process that actually provides three dimensional muscle tissue. The same is valid for stem cells and somite cells. The Example provided shows in detail how this technology can be applied for these cells. We refer to a number of articles which provide detailed descriptions leading from somite cells and stem cells to differentiated cells like muscle cells, blood cells and blood vessels (Mummery et al 1990 and 1993, Slager et al 1993, van Stekelenburg-Hamers 1995 and thesis 1994). The content of these cited documents is incorporated by reference. We also point out that the method for producing meat can also comprise culture of a number of other cell types such as blood in addition to the muscle cells. The methods indicated in the cited articles are capable of upscaling to industrial scale without requiring substantial changes in methodology. Where application of antibiotics is mentioned in the examples and the cited references these can be omitted and the use of sterile conditions e.g. in a clean room can be introduced instead. The process according to the invention thus offers the possibility of meat production free of hazardous substances. Such a hazardous substance is an antibiotic e.g. penicillin. It is also intended to carry out the method according to the invention in absence of antibiotic. It is also intended to carry out the method in absence of hormones. Specifically in absence of corticosteroid hormones like dexamethasone, cortisol and hydrocortisone. Subsequently the resulting food product is thus also free of hazardous antibiotics and hormones. Specifically the advantage is seen for the absence of steroid hormones as these are exceptionally hazardous. The process is also to be carried out in absence of hyaluronic acid derivatives. Preferably the process is also carried out in absence of bovine serum with a view to circumventing any contamination with BSE or mad cows

disease causing viral material.

Alternatively the three dimensional culture is contemplated as occurring on a matrix as described in a manner analogous to that disclosed. The muscle cells to be used in such a new procedure can in turn be obtained from procedures as illustrated in the examples. The same

is valid for stem cells and somite cells. The matrix can be collagen or a synthetic substrate. If the latter is non-digestible, it should be removed during work up to the edible end product. In an alternative embodiment culture can occur in suspension or by producing monolayers and subsequently compacting a number of grown monolayers to a three dimensional structure. The technical details for these embodiments can be derived from literature available to the person skilled in the art from the medical literature for monolayer cultivation of muscle cells in vitro. The process naturally needs to be upscaled to industrial scale to render it a suitable process for food production.

In a preferred embodiment the cells to be cultured are selected from somite cells which are cells in the predifferentiated stage preceding muscle cells. The somite cells can be taken from tissue in a manner known per se. The regenerative power of such cells is larger than that of already differentiated cells and as such could ensure a higher production per cell used in the original culture. Stem cells form an alternative source of cell material.

As such the invention is not merely directed at a novel production process but at a novel food product, consisting of in vitro produced animal cells in a three dimensional form i.e. comprising multiple cell layers of animal cells in three dimensions, said meat product being free of fat, bone, tendon and gristle and preferably free of growth hormones in non physiological amounts, said cells being selected from muscle cells, somite cells and stem cells. As it is free of tough products, the product is easier to consume for the ageing population.

The product retains the structure and flavour of lean meat without the ensuing animal suffering or concomitant ethical, religious, economical and environmental problems associated with regular production of meat comprising food products as detailed above in the introduction. The resulting product is thus healthier and also has better texture than standard meat. The subject product can be used to replace current animal produced meat as food or component of food products such as sausages, processed meats, soups, stews, purees, granulated protein products and regular meat cuts.

The novel product according to the invention can be produced in unit sizes enabling ease of transport and storage. The product can be factory produced in a largely automated process as is common in known food processing processes requiring cell culture. The final product exiting from the factory can be packaged in situ either in fresh, precooked, 5 dried or frozen form analogous to meat obtained from live animals without requiring a step of the removal of debris and offal. It can be discerned from other current meat produce due to the lack of fat, bone, tendon and gristle and the regular and uniform composition of the muscle cells. the required consistency for application can be achieved by denaturing the protein once it has been produced e.g. by cooking, boiling etc much in 10 the manner analogous to treatment of the proteinaceous substance of eggs i.e. protein solidification steps.

In a preferred embodiment the novel food product according to the invention can further comprise nutritional additives such as vitamins and minerals to further enhance the nutritional value thereof. Care should be taken to keep the resulting product 15 as natural as possible, thus additives should be used within physiological boundaries. Due to the production process of the novel product it is simple in a controlled manner to enhance the nutritional value by adding the desired added nutrient or compound to the growth medium in a form accessible to the cell.

The cells can be cultured in admixture with the muscle cells, somite cells or 20 stem cells or be cultured separately and added in an amount according to choice to the cultured muscle cells prior to processing of the cell culture of muscle cells to the final product. The processing to the final product will be different depending on which method of cell culture is selected. The process comprises bringing the cell culture to a solid state and texture comparable with that of in vivo derived meat. The exact 25 methodology for working up from the cell culture will be a matter of choice dependent on the destination of the final meat product and whether it is to be fresh, dried, frozen, pre or partially cooked, pickled or smoked etc. and is to be incorporated in a food product such as soup, stew, sausage, spread, puree e.g. baby food, biscuit or dried granules, tablet or powder etc. The advantage is that such further processing can easily 30 be combined with the original process of producing the animal cell ingredient of the finished food product and does not require the traditional steps of offal removal, deboning and removal of gristle, tendon and/or fat. The cells can be derived from mammals, e.g. cow, sheep, goat, pig, deer, rabbit, hare, whale, kangaroo; birds, e.g.

chicken, goose, pheasant, duck, ostrich and partridge; reptiles, e.g. frog, turtle, crocodile; fish, e.g. tuna, eel, cod, sole, shark and herring; and shellfish, e.g. oyster, crab, langoustine and shrimp. Mixtures or combinations of the above can also be made.

For initiating this industrial meat/fish vital cell culture method according to the invention per animal sort only a small number, preferably intracellular 100% pure embryo muscle or somite cells are required. These initial cells can be obtained from specially selected donor animals for this purpose. The donor animals are kept under very strict environmental conditions, i.e. in quarantine, in clean rooms and with feeding conditions etc. during a longer period under the directions of a team consisting of i.a. a biologist, a surgeon, a histologist, a veterinarian surgeon, a computer programmer, a chemist, a bacteriologist, a pharmacologist, a technical engineer and any other scientific staff. For these initial cell cultivating procedures it is not necessary that the donor animals be killed or slaughtered. These selected and supervised donor animals will be treated in an animal friendly manner and by detoxification treatment and correct feeding the current serious environmental burden on animal cells will be combatted which will improve the general degree of health, resistance and germination strength in an optimal manner. For this objective computer regulated cell quality control e.g. in combination with the aid of an electron microscope can be used. In addition, the donor animals should regularly be checked by veterinarians with serological and bacterial assays for infectious diseases such as salmonella infection, brucellosis, listeriosis, leptospirosis, toxoplasmosis and Q-fever and tuberculin tests. By means of this specially regulated supervision the muscle/somite cells can continually progress with cell division for meat production outside the intact organism. In particular, the composition of the feeding medium is of large importance for this cell division for 100% pure meat/fish production. G.M. Heally and associates developed a feeding medium 858 consisting of 62 ingredients, namely 20 amino acids, 12 vitamins, 7 co-enzymes, 2 lipid sources, 5 nucleic acid products, 3 antibiotics and serum. This medium resulted in a 10-fold increase of the number of cells. Such feeding media and others can be used for the culture of the meat vital cells according to the invention. Naturally adaptations of such media can be suitably used. In addition it is important to regulate any undesired changes of pH and oxygen in a continuous manner by computer. The muscle/embryo and somite cells growth required for this 100% pure meat production according to a new unique industrial cultivation method and can be computer regulated and quality controlled to

provide a 100% pure protein product. Doctor Robin Holliday of the National Institute for Medical Research in London is of the opinion that the dying of (culture) cells should be attributed to errors. Dr. Holliday carried out experiments in this regard with fungi. These fungal cell cultures which normally practically have eternal life started to age quickly 5 when errors were introduced into the fungal cell cultures. Fungal cultures however where these errors were not introduced could live on endlessly with their vital culture cells. Current meat and fish comprise material with the same errors as introduced in the fungal cells because proteins of insufficiently checked slaughter cattle exhibit a strong increase 10 in erroneous, less than 100% normal proteins due to their bad food and maintenance conditions. So current cattle and the meat/fish producing animals will i.a. by wrong environmental influences increasingly comprise meat or fish that is produced by the current methods with bad proteins. This will result in an increase in more bad proteins being introduced into the human metabolism i.e. result in an increased aging, a reduced 15 immune system and increased susceptibility for breading of disease. Thus, lending credence to the adage "you are what you eat". With increasing age (after the embryo state) under influence of the current negative environmental influences, the cells of living animals will become increasingly less healthy, for example by poisoning of the tissue fluid i.e. the internal medium of muscle cells of cattle. The molecular biologist Dr. Robin Holliday clearly thus sees the cause of disease, fatigue increased aging and 20 negativism in the cellular autoimmunization processes taking place. The findings of Dr. Holliday can be found in Nature 221, 1969, p. 1234-1238. Also Dr. L. J. Orgel of the Salk Institute in San Diego published in Proc. US Nat. Acad., set 49, 1963, p. 517 a connection between increased negative factors and increased aging with bad proteins. Theoretically it means that the number of "bad proteins" increases by mistakes in the 25 cattle in the growing process. From these erroneous proteins a large number of erroneous proteins will be derived which will in turn mean that the total metabolism of cells will no longer function properly and the erroneous proteins will result in development of specific antibodies with all the consequences thereof to the animal and the consumer of such animal product. Recent newspaper articles also indicate that the contamination of 30 ground and water in the environment to which cattle are exposed are also resulting in toxicological compounds having a profound effect. Prof. Dr. P. Schepens, a professor in Antwerp indicated that drinking water in the lower lying areas such as the Netherlands are contaminated with carbondisulphide which apparently causes a disease similar to aids

in cattle. In a number of cows lately mysterious diseases have been found, such as dysfunctional growth, skin problems, swollen joints and equilibrium problems. The cause could be due to intensive oil and gas winning which resulted in contamination of the salt swamp lands lying approximately 50 cm to 5 m below sea level. During a number of 5 decades upward water streams have brought oil up to the surface. According to Dr. G. Counotte, a toxicologist at the Health Service for Animals approximately 70% of the surface water is contaminated. According to Dr. Counotte within a couple of years the cattle to the West of Utrecht in the Netherlands will need to be destroyed. Thus, it is of the utmost importance that the industrial meat/fish production according to the invention 10 having a 100% pure protein composition is implemented as soon as possible within the near future to prevent the continued environmental burden and to enhance the current health of the population and in particular to enhance the health of future generations.

The applicants of this patent are committee members of Foundation Worldwide Medical 15 Water Orientation. This foundation has as objective cooperation with the United Nations and other aid organisations to enable the provision of good pure drinking water in developing countries in refugee camps and i.a. in the ghettos of large cities in South America etc. In addition, the foundation has as task enabling grounds in areas where it is 20 too warm and where insufficient rain falls to be rendered useful for agricultural by means of a new system, in order to attain better health and more prosperity. Any revenues to be realized upon exploitation of this patent are also destined to be used to finance the above-mentioned objectives of the foundation. The chairman of the foundation, W.F. van Eelen, already had the idea of this patent application in view of the enormous importance of the exploitation of the possibilities mentioned in the patent in 25 1950 during his studies and followed the scientific developments for many years thereafter in order to arrive at the subject matter described in the patent application. In this respect and in view also of the current environmental problems knowing that his important idea of 47 years ago had now become technically and scientifically exploitable he already deposited the idea at the roots of the subject patent application under a 30 secrecy agreement with the Inland Revenue Service of The Netherlands in Rijswijk for purposes of establishing date of conception at least as early as March 3, 1995. Proof of this is presented as enclosure 1. The translation of the deposited text is as follows:

"The industrial production with new techniques on a large scale i.a. using elements of

laboratory tissue cell culture method of all 100% pure meat and fish sorts with complete maintenance of exterior, taste facets and character, thereby rendering the keeping of cattle (fish) and the slaughtering (catching) thereof, i.a. as economically too costly - superfluous. The consumer is provided with an in particular more tasty and more tender (where necessary) also cheaper 100% pure meat and fish. The computer regulated growth production also renders this meat and fish in all existing variations more healthy for human consumption, i.a. by the complete lack of hazardous additives and any negative environmental influences. (Positive consequences with regard to public health).  
5 I.a. by the availability of extensive grazing ground which is now required for cattle cultivation, agricultural possibilities are extended enormously which can be of interest  
10 i.a. for the environment and in addressing the world food shortage".

## EXAMPLES

15

### MATERIALS MENTIONED IN EXAMPLES

Albumin bovine (BSA) BDH 1 g/vial. Cat. Nr. 44004

20 Amicon ultrafiltration cell 50 ml. Model 52. Amicon Comp. Lexington Mass. 02173.  
Brown sterilizer control tubes for ovens at 160° C Type 3.  
Green Spot. (Lameris, Biltstraat 449, 3572 AW Utrecht).

25 Chick embryo extract 20 ml 50% in Earle's Balanced Salt Solution. Flow. Cat. Nr. 28-501-46. (Keeps ca. 6 months at -70°C).  
Collagenase 250 mg/flask. Sigma Cat. Nr. C-2139.  
(Store at -20°C).

30 Diaflo ultrafiltration Membranes 25 PM 10 43 mm.  
Amicon Comp. Lexington Mass. 02173.

Dimethylsulphoxide (DMSO)) Analar BDH Nr. 10323 500 ml/bottle.

Dulbecco's Minimal Essential Medium (DMEM) x 10 without L-glutamine 500 ml/bottle. Gibco Cat. Nr. M 07-2501.

Ethylenediamine tetraacetic acid 100 g/bottle.

5

Dulbecco's Minimal Essential Medium (DMEM) x 10 without L-glutamine 500 ml/bottle. Gibco Cat. Nr. M 07-2501.

Ethylenediamine tetraacetic acid 100 g/bottle

10 BDH Cat. Nr. 28021-2Q.

Falcon Tissue Culture Flask. 25 cm<sup>2</sup> growth area, two position cap. 500/case. Becton-Dickinson Oxnard Cal. Cat. Nr. 3013.

15 Foetal Calf Serum (FCS) Gibco Cat. Nr. 629 HI 100 ml/bottle.

(Keeps ca. 6 months at -70°C).

Fungizone 50 mg/flask. Squibb.

L-glutamine 25 g/bottle. Sigma Cat. Nr. G-3126. (Store at 4°).

Horse serum (HS) Gibco Cat. Nr. 605 HI. 100 ml/bottle.

20 (Keeps about 6 months at -70°).

Hydrochloric acid, crude, ca. 30% 1 l/bottle.

Millex Millipore Filter Units, disposable.

0.22 µm: Cat. Nr. SLGS-025-OS

25 0.45 µm: Cat. Nr. SLHA-025-OS

50/box.

Millipore Sterifil-D units, disposable. Cat. SGFS-047-LS

Millipore Comp., Bedford (Mass.)

Nigrosine 25 g/bottle. BDH Cat. Nr. 34058

30 Nylon filter gauze, mesh width 20 and 50 µm. Minimal order 100 x 100 cm. Nyta Nylon Seidengaze. Schweizerische Seidengazefabrik CH-9425 Thal/Switzerland.

Parafilm M. American Can Comp. Dixie/Marathon greenwich CT 06830. 4 in x 125 ft/roll.

Pasteur Capillary Pipettes. Short size 150 mm.

Penicillin-NA. 1.000.000 U/flask.

Petri dishes, sterile, disposable. 500/case. 60 x 15 mm.

Lux Scientific Corp. 1157 Tourmaline Drive Newbury Park, Calif. 91320. Cat. Nr. 5220.

5 Phenol red pH=6.8-8.4 (yellow-red). British Drug House Nr. 20090. 5 g/bottle.

Phosphate Buffered Saline x 1 (PBS). 500 ml/bottle.

Flow Cat. Nr. 18-604-54.

Sodium Azide 25 g/bottle. Sigma S 2002 (Explosive!)

10

Sterilin containers 20 ml. 400/case. Printed Label. Sterilin Ltd., Teddington, Middlesex U.K., Cat. Nr. 128 B.

Streptomycin sulphate. 1 g/flask.

Tuberculin.

15

Trypsin 1: 250 Difco 100 g. Difco lab. Detroit Cat. Nr. 0152-15

Water, bidistilled, 100 ml/bottle.

20 **1. DISSOCIATION OF MUSCLE TO OBTAIN STARTING MATERIAL**

This can be achieved by way of example in any number of manners, e.g.

25

- A. Rinse a muscle biopsy a few times in PBS solution
- B. Fill a sterilin container with dissociation solution up to 20 ml
- C. Introduce the muscle biopsy into a sealable container with a wide neck and add 7 ml dissociation solution.
- D. Put this container in a water bath and shake it at 37°C such that the biopsy moves through the medium
- 30 E. After exactly 15 minutes pour off the liquid into 7 ml 10% FCS in DMEM
- F. Add 7 ml again to the biopsy and disrupt the biopsy somewhat by pulling, however, do not divide into parts

G. Reintroduce into the bath at 37°C for 15 minutes and repeat the same procedure twice.

5 H. After which add the three containers with 14 ml (7 ml dissociation solution and 7 ml 10% FCS) to each other and centrifuge at 1200 rpm for 5 minutes

I. Dissolve the pellet in 4 ml 20% FCS and in a 75 CM culture flask and give the cells 24 hours to attach

10 J. Rinse after 24-48 hours and replenish with 20% FCS and proceed to muscle cell culture either as trabecular matrix approach, in suspension approach or in monolayer approach.

## 2. CULTURE ON TRABECULATED MEDIUM

15 The muscle cells that can be obtained in any of the procedures of 1 can subsequently be used in a culture of cells in trabeculated medium. The trabeculated medium will preferably having a poresize of 25-200  $\mu\text{m}$ , preferably 30-150  $\mu\text{m}$ . Dry collagen sponges, for example of a thickness of 2-20 mm can be fabricated in a freeze-drying process from insoluble native collagen type I fibres of bovine origin.

20 Subsequently the sponges can be coated with  $\alpha$ -elastin hydrolysate from bovine ligamentum nuchae in a concentration of 3 w/w%. It is also known from the state of the art that recombinant collagen or collagen derivatives can be produced, naturally these can be used in the production process if so desired. The cell populations can be seeded into the collagen sponges at a concentration of  $10^5$  viable cell/cm<sub>2</sub>. This can be placed on a

25 flat container e.g. a petri dish or in a cylindrical container into which the matrix is placed in a folded manner or it can be rolled into a three dimensional structure through which medium is percolated. The medium can be refreshed as often as necessary, e.g. one or two times a week. Such a system can be automated. There is less risk of infection and cells that are not continually being replated will have a longer replication life time

30 with the concomitant advantages thereof. The container with myoblast containing sponges can be maintained at culture conditions allowing attachment to the matrix fibres and by incubating. The sponges in growth medium at the required temperature growth of the cells on the cell matrix can occur. Analogously other trabeculated matrix material can

be used. this can be polyurethane, polylactic acid, chitine for example. The cells can either be used together with the matrix if an edible matrix is used or be removed from the matrix as part of the production process. Once confluence has been reached the culture medium must be removed, the meat product is ready to be used and does not require further processing steps as such. If thin layers are produced, a number of layers may be combined to achieve the desired product thickness. Upscaled and automated procedures analogous to the above are preferred embodiments.

### 10 3. CULTURE IN SUSPENSION

The muscle cells that can be obtained in any of the procedures of 1 can subsequently be used in a culture of cells in suspension. Upscaled and automated procedures are preferred embodiments.

15 Cells can be added to microcarriers such as Cytodex 1 and 3 beads or dorma cells. The attachment of the cells can occur by contacting cells from a monolayer culture onto the microcarriers when these are put on top of the monolayer. In a rotating system the growth of the cells on the microcarriers can take place while the microcarriers are kept 20 in suspension. In a suitable embodiment cells and carriers can be combined in a ratio of 10:1 in 25 ml of growth medium (1 mg carriers/ml) in culture flasks. The flasks can be gassed with 5% CO<sub>2</sub> and sealed. After adjustment of the pH of the medium to 7,2 cells and carriers can be transported into 25 ml test tubes which are subsequently sealed. These test tubes can be rotated at 7 rpm on a Cel-Gro Rotator (Lab Line Instruments, 25 Inc. IL) in a cultivation chamber at 37°C. Medium can be refreshed twice a week after settling down of cells and carriers. It is also possible to carry out the experiment in a cell culture fermenter vessel comprising 2 mg/ml; 0,8 x 10<sup>3</sup> carriers/ml of for example Cytodex 3 microcarriers and inoculating these in a ratio 9:1 in 1.2 litres of growth medium, e.g. 10% FCS. The carriers can be kept in suspension with a cell lift impeller 30 at an agitation speed of 22-25 rpm. The temperature of 37°C and pH of 7.2 and the dissolved oxygen, amount of 50% can automatically be controlled by the cell culture system (Celligen, New Brunswick Scientific Co., Inc., Edison, NJ). Medium can be refreshed batchwise (0,5 l) twice a week after cells and carriers have settled. After

growth the cells can be detached from the microcarriers, for example by incubation in 0.02% EDTA and 0.005% trypsin in PBS-3. Cells can subsequently be separated from the carriers by filtration over 150  $\mu$ m screen in a Collector, tissue sieve (Bellco Glass, Inc., Vineland, NJ). Cells in the filtrate can be harvested as cellpellets after 5 centrifugation or used for repeated culture.

The attachment to cytodex 1, cytodex 3 and dorma cells can be seen to occur almost immediately after cells and carriers are combined. Subsequently the cells spread on the microcarriers within a couple of hours. In both the suspension in the rotating system as 10 in the fermenter system good growth is obtained and growth to confluence or near confluence was also obtained relatively quickly. When confluence or near confluence on microcarriers is reached detachment of parts of the cell sheet from the microcarriers occurs. In the stationary system this results in reattachment to the culture flasks surface. In the rotating systems these unattached cells can be harvest at medium refreshment and 15 can be used for replating in culture flasks or be harvested for use in the production of the food product. In the rotating test tubes almost complete confluence is reached on all the carriers resulting in a cell density a lot higher than on the culture flasks surface. The results in the fermenter system prove that enormous quantities of cells can be obtained in a very short time. In the fermenter system it also is easier to maintain large quantities of 20 cells as opposed to culture flasks in view of being less labour intensive with less risk of contamination and requiring less space or storage than for the flasks. Depending on the cell type, the media and the growth conditions can be optimized in a manner clear to a person skilled in the art. Higher agitation speed and carrier concentrations during inoculation can for example result in a more even distribution of cells over the carriers. 25 Extra addition of amino acid vitamins, glucose and attachment factors or medium refreshment by perfusion could also improve the results. Scaling up to a 2.5-l vessel is expected to provide cell numbers in the order of  $10^9$ .

30 **4. CULTURE IN MONOLAYER**

**4.1. MYOBLAST CULTURE IN MONOLAYER**

Procedure:

- A. Contact the myoblast culture with FCS fungizone medium.
- B. Place flask into incubator without shifting the content.
- 5 C. Replace medium after 72 hours if yeast or fungus remain and use FCS fungizone for another 72 hours. Use 20% FCS for this treatment.
- D. Incubate at 37°C, 5% CO<sub>2</sub> with a relative moisture of 98%. Myoblast proliferation occurs at a rate wherein doubling occurs in three to four days. Do not let the myoblast culture become confluent. At a maximum let it grow 10 to 60 to 70% confluence then trypsinate immediately.
- E. To trypsinate seed a 25 cm<sup>2</sup> Falcon flask with 50-80.000 myoblast in 4 ml FCS medium.
- F. When the cells have been proliferated then preplate them in 75 CM culture flask, i.e. simply trypsinate and replace in a 75 CM flask and return to the 15 incubator.
- G. After exactly 20 minutes the container can be carefully put upstanding and the supernatant can be transferred to a clean 75 CM flask. In this treatment most of the fibroblasts which are always present in a myoblast culture will remain behind in the first flask because these fibres adhere quicker than the 20 myoblasts. These fibroblasts can disturb differentiation and for this reason must be removed. Please note the fibroblasts will only be present in the first sample, subsequent cycles of proliferation will be already free of fibroblasts and will not require this step.
- H. Upon sufficient density (approximately 70% confluent) the cells can be 25 replenished with differentiation medium which will enable formation of myotubes, an irreversible process that takes place approximately after 6-8 days. These myotubes cannot be trypsinated and should not be replenished during the differentiation. These myotubes only remain for a few days and than collapse.

30

**4.2. HARVESTING OF MONOLAYER CULTURES**

**I. Preparation:**

- A. Switch on laminar flow cabinet before onset of procedure
- B. Clean rear, sides, and bottom of flow cabinet with alcohol 96%
- C. Put in the cabinet:

5

- 1. Enough weighed and labelled Eppendorf cups
- 2. Beaker with ice containing:
  - a. Eppendorf cups, placed there immediately before each

10 harvest.

- b. 1 Sterilin container with 10-15 ml PBS (Flow)
- 3. Bottle of 500 ml of PBS (need not be or stay sterile)
- 4. Rubber "policeman"
- 5. Kitchen knife
- 6. 100  $\mu$ l pipette
- D. Place beside the cabinet:
  - 1. Yellow pipette tips (for 100 $\mu$ l pipette)
  - 2. Syringe with 70% alcohol and a tissue, for cleaning the policeman after each use.

20

**II. Procedure.**

- A. Take a small (25 cm<sup>2</sup>) Falcon bottle from the incubator  
Screw the cap on tightly.
- B. If necessary, examine it under the microscope, or take a  
25 picture of it.
- C. Using the kitchen knife, snap off the top side of the Falcon  
bottle.
- D. Remove PBS and any plastic bottle fragments.
- E. Remove the medium by suction, and add 3 ml of PBS from the  
30 500 ml bottle, leaving a 10 ml pipette standing in the bottle.
- F. Remove and replace the 3 ml of PBS.
- G. Replace and remove 3 ml of PBS.
- H. Add 400  $\mu$ l of PBS (2 x 200  $\mu$ l, using the 200  $\mu$ l pipette from

the Sterilin container.

- I. Using the rubber policeman, scrape PBS and cells into corner.
- J. With same blue tip, pipette the cell-PBS suspension into the Eppendorf cup in the beaker on ice.
- 5 K. Put the Eppendorf cup into the freezer, immediately and upright.
- L. Discard pipette tip and remnant of the Falcon bottle.
- M. Cleanse the policeman with a tissue soaked in 70% alcohol.
- N. Replace ice in beaker if necessary.
- 10 O. After harvesting all bottles, rapidly weigh all the Eppendorf cups, and store them in the -70° freezer.

A monolayer culture will be removed from the growth medium and a number of layers will be brought together and contacted with each other thereby forming a three dimensional food product per se or ingredient for a food product. A number of monolayers can e.g. be compacted thereby providing the finished product. In the case of a suspension the growth medium can be removed or the solid part of the cell culture comprising the cells can be removed. The exact methodology to be applied will depend on the content of the culture medium and the desirability of the final product comprising components of said growth medium other than the cultured cells.

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10

5. Van Stekelenburg-Hamers A.E.P. thesis entitled "Developmental Potency in early bovine embryo's of October 5, 1994.

1. A process for production of a meat product said process comprising the culturing in vitro of animal cells in medium free of hazardous substances for humans on an industrial scale thereby providing three dimensional animal muscle tissue suited for human and/or animal consumption, followed by further processing steps of the cell culture to a finished food product analogous to known processes for meat comprising food products without requiring deboning, removal of offal and/or tendon and/or gristle and/or fat, preferably said meat product comprising solidified cell tissue, said cells being selected from muscle cells, somite cells and stem cells.
2. A process according to claim 1 wherein the cell culture is carried out using a culture vessel with a volume larger than 5 litres, preferably larger than 1000 litres, more preferably larger than 5000 litres.
3. A process according to any of the preceding claims wherein the culture step is carried out using a trabeculated three dimensional matrix for the cell growth using suitable medium for muscle cell growth, somite cell growth or stem cell growth under suitable conditions for said growth and wherein the matrix is optionally later removed in a manner known per se.
4. A process according to any of the preceding claims wherein the cell culture is carried out in suspension using suitable medium for muscle cell growth, somite cell growth or stem cell growth under suitable conditions for said growth in a manner known per se for cell culture.
5. A process according to any of the preceding claims wherein the cell culture is carried out in monolayer cell culture using suitable medium for muscle cell growth, somite cell growth or stem cell growth under suitable conditions for said growth, followed by contacting a number of said layers thereby forming a three dimensional food product comprising multiple layers of muscle cells, somite cells or stem cells in three dimensions.
6. A process according to any of claims 1-4 wherein the resulting cells are

compacted to a three dimensional meat product.

7. A process according to the preceding claims, wherein the growth medium consists merely of physiologically acceptable components.

5

8. A process according to any of the preceding claims, wherein the cultivation conditions of the cells comprise contact with O<sub>2</sub> and CO<sub>2</sub> e.g. under approximately 5% CO<sub>2</sub> and at body temperature for the in vivo environment of the animal cell being cultured in general at approximately 37°C and under sterile conditions.

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9. A process according to the preceding claims, wherein the process further requires addition of nutritional and/or flavour providing compounds e.g. salts and/or spices, such nutrient being added to the growth medium in a form accessible either to the cell or in a form such that it is incorporated in the final product during the processing of the cell culture to the final product e.g. in the form of a precipitate mixed in with the cells as such or else incorporated within the cells themselves.

20

10. A process according to any of the preceding claims, wherein the growth medium comprises growth factors for the cells, said growth factors being within physiological limits.

20

11. A process according to any of the preceding claims wherein the original cells used in the culture are muscle cells.

25

12. A process according to any of the preceding claims wherein the original cells used in the culture are stem cells.

13. A process according to any of the preceding claims wherein the original cells used in the culture are somite cells.

30

14. A process according to any of the previous claims comprising a further processing step of the cell culture selected from solidification, drying, freezedrying, freezing, pickling, boiling, cooking, smoking and packing.

15. A process according to the previous claim further comprising a food processing step such as processing to any of the following consumption forms a meat substitute, soup, pie, sausage, spread, stew, baby food e.g. puree, biscuit, dried granules, tablet, capsule, powder or other solid or liquid forms in a manner known per se for producing meat comprising products derived from in vivo meat production.

5  
16. A meat product, consisting of in vitro produced animal cells in a three dimensional form i.e. comprising multiple cell layers of animal cells in three dimensions, said meat product being free of fat, tendon, bone and gristle, said cells being selected from muscle cells, stem cells or somite cells.

10  
17. A meat product derivable from a process according to the claims 1-15.

15  
18. A meat product according to any of the claims 16 or 17 free of growth hormones in amounts hazardous for consumption.

20  
19. A meat product according to any of the claims 16-18 further comprising nutritional additives such as vitamins and minerals to further enhance the nutritional value thereof.

20  
20. A meat product according to any of the claims 16-19 in a solid state and texture comparable with that of in vivo derived meat.

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21. A meat product according to any of the claims 16-20 in a form selected from fresh, dried, frozen, pre or partially cooked, pickled or smoked as such or incorporated in a further processed food product such as soup, stew, sausage, spread, puree e.g. baby food, biscuit, dried granules, tablet, capsule, powder or other solid or liquid form.

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22. A meat product according to any of the claims 16-21 in a unit form e.g. of at least 50 grams, preferably at least 100 grams.

23. A meat product such as soup, stew, sausage, spread, puree e.g. baby food, biscuit dried granules, tablet, capsule, powder or other solid or liquid form comprising

the food product according to any of claims 15-22 as ingredient.